

Appl. No. 10/086,972
Amdt. dated May 2, 2005
Reply to Office action of February 2, 2005

Amendments to the Specification:

Please replace the title on page 1, line 2-3, and page 32, line 3-4, as follows:

~~NOVEL USES OF MAMMALIAN OX2 PROTEIN AND RELATED REAGENTS~~
USES OF OX2 PROTEIN TO TREAT NEURODEGENERATIVE DISORDERS

Please replace the paragraph on page 1, lines 5-8, as follows:

~~The present application is a conversion to a US Utility patent application of U.S. Provisional Patent Application USSN 60/129,124, filed March 13, 1999, which is incorporated herein by reference. The present application is a divisional application of then copending USSN 09/547,432, filed April 12, 2000, now abandoned, which claims benefit of US provisional application USSN 60/129,124, filed March 13, 1999, each of which is incorporated herein by reference.~~

Please replace the paragraph on page 19, lines 31-36, as follows:

~~For in vitro or in vivo biological assays, OX2 or OX2-E tag are produced, e.g., in large amounts with transfected COS-7 cells grown in RPMI medium supplemented with 1% Nutridoma HU (Boehringer Mannheim, Mannheim, Germany) and subsequently purified. Adenovirus expression systems may be used. For in vitro or in vivo biological assays, OX2 or OX2-E-tag are produced, e.g., in large amounts with transfected COS-7 cells grown in RPMI medium supplemented with 1% NUTRADOMA HU supplement (Boehringer Mannheim, Mannheim, Germany) and subsequently purified. Adenovirus expression systems may be used.~~

Please replace the paragraph on page 20, lines 6-10, as follows:

~~Inbred Balb/c mice are immunized, e.g., with 1 ml of purified OX2 emulsified in Freund's complete adjuvant on day 0, and in Freund's incomplete adjuvant on days 15 and 22. The mice are boosted with 0.5 ml of purified OX2 administered intravenously. Inbred BALB/c mice are immunized, e.g., with 1 ml of purified OX2 emulsified in Freund's complete adjuvant on day 0, and in Freund's Incomplete adjuvant on days 15 and 22. The mice are boosted with 0.5 ml of purified OX2 administered intravenously.~~

Appl. No. 10/086,972
Amdt. dated May 2, 2005
Reply to Office action of February 2, 2005

Please replace the paragraph on page 20, lines 11-31, as follows:

~~Hybridomas are created, e.g., using the non-secreting myeloma cells line SP2/0-Ag8 and polyethylene glycol 1000 (Sigma, St. Louis, MO) as the fusing agent. Hybridoma cells are placed in a 96-well Falcon tissue culture plate (Becton Dickinson, NJ) and fed with DMEM F12 (Gibco, Gaithersburg, MD) supplemented with 80 µg/ml gentamycin, 2 mM glutamine, 10% horse serum (Gibco, Gaithersburg, MD), 1% ADCM (CRTS, Lyon, France) 10^{-5} M azaserine (Sigma, St. Louis, MO) and 5×10^{-5} M hypoxanthine. Hybridoma supernatants are screened for antibody production against OX2, e.g., by immunocytochemistry (ICC) using acetone fixed OX2 transfected COS-7 cells and/or by ELISA using OX2 purified from COS-7 supernatants as a coating antigen. Aliquots of positive cell clones are expanded for 6 days and cryopreserved as well as propagated in ascites from pristane (2,6,10,14-tetramethylpentadecane, Sigma, St. Louis, MO) treated Balb/c mice who had received on intraperitoneal injection of pristane 15 days before. About 10^5 hybridoma cells in 1 ml of PBS are given intraperitoneally, and 10 days later, ascites are collected from each mouse.~~ Hybridomas are created, e.g., using the non-secreting myeloma cells line SP2/0-Ag8 and polyethylene glycol 1000 (Sigma, St. Louis, MO) as the fusing agent. Hybridoma cells are placed in a 96-well FALCON tissue culture plate (Becton Dickinson, NJ) and fed with DMEM F12 media (Gibco, Gaithersburg, MD) supplemented with 80 µg/ml gentamycin, 2 mM glutamine, 10% horse serum (Gibco, Gaithersburg, MD), 1% ADCM (CRTS, Lyon, France) 10^{-5} M azaserine (Sigma, St. Louis, MO) and 5×10^{-5} M hypoxanthine. Hybridoma supernatants are screened for antibody production against OX2, e.g., by immunocytochemistry (ICC) using acetone fixed OX2 transfected COS-7 cells and/or by ELISA using OX2 purified from COS-7 supernatants as a coating antigen. Aliquots of positive cell clones are expanded for 6 days and cryopreserved as well as propagated in ascites from PRISTANE (2,6,10,14-tetramethylpentadecane, Sigma, St. Louis, MO) treated BALB/c mice who had received on intraperitoneal injection of pristane 15 days before. About 10^5 hybridoma cells in 1 ml of PBS are given intraperitoneally, and 10 days later, ascites are collected from each mouse.

Appl. No. 10/086,972
Amdt. dated May 2, 2005
Reply to Office action of February 2, 2005

Please replace the paragraph beginning on page 20, line 32 through page 21, line 4, as follows:

~~After centrifugation of the ascites, the antibody fraction may be isolated by ammonium sulfate precipitation and anion-exchange chromatography on a Zephyr-D silicium column (IBF Sepracor) equilibrated with 20 mM Tris pH 8.0. Proteins are eluted with a NaCl gradient (ranging from 0 to 1 M NaCl). 2 ml fractions may be collected and tested by ELISA for the presence of anti-OX2 antibody. The fractions containing specific anti-OX2 activity are pooled, dialyzed, and frozen.~~
After centrifugation of the ascites, the antibody fraction may be isolated by ammonium sulfate precipitation and anion-exchange chromatography on a ZEPHYR-D silicium column (IBF Sepracor) equilibrated with 20 mM Tris pH 8.0. Proteins are eluted with a NaCl gradient (ranging from 0 to 1 M NaCl). 2 ml fractions may be collected and tested by ELISA for the presence of anti-OX2 antibody. The fractions containing specific anti-OX2 activity are pooled, dialyzed, and frozen.

Please replace the paragraph on page 21, lines 7-36, as follows:

~~OX2 knockout (KO) mice were made essentially according to the procedure described by Galli-Taliadéro, et al. (1995) J. Immunol. Methods 181:1-15; Kömer, et al. (1997) Eur. J. Immunol. 27:2600-2609; and Lemckert, et al. (1997) Nucl. Acids Res. 25:917-918. In short, a C57BL/6 genomic library was screened using a PCR fragment of the mouse OX2 cDNA as a probe. The isolated genomic clone contained an insert of about 16 kb from which a 9.5 kb SalI fragment was sub-cloned into pBluescript. This clone contained part of intron I, exon II (encoding the signal peptide), intron II, exon III (encoding the V-like Ig domain), intron III, exon IV (encoding the C2-like Ig domain), and part of intron IV. From this clone a targeting construct was created by replacing an NcoI fragment encoding the C-terminal part of the V-like Ig domain with the Neomycin cassette and shortening the upstream part of the clone so that it contained only the 3' part of the exon encoding the signal peptide. An ES cell line derived from C57BL/6J mice (Bruce 4; see Galli-Taliadéro, et al. (1995) J. Immunol. Methods 181:1-15 and Lemckert, et al. (1997) Nucl. Acids Res. 25:917-918) was transfected by electroporation, and G418-resistant colonies were isolated and screened for homologous recombination by PCR and Southern blotting. One homologous~~

Atty. Dkt. DX0936KB

Page 4 of 12

Appl. No. 10/086,972
Amdt. dated May 2, 2005
Reply to Office action of February 2, 2005

~~recombinant out of 1,000 clones was isolated and used to create chimeric mice. See Lemckert, et al. (1997) Nucl. Acids Res. 25:917-918. Male chimeras were bred with female wild type C57BL/6J mice and the offspring with black coat color (indicating germ-line transmission) were screened for the presence of the targeted allele. OX2 knockout (KO) mice were made essentially according to the procedure described by Galli-Taliadoros, et al. (1995) J. Immunol. Methods 181:1-15; Kömer, et al. (1997) Eur. J. Immunol. 27:2600-2609; and Lemckert, et al. (1997) Nucl. Acids Res. 25:917-918. In short, a C57BL/6 genomic library was screened using a PCR fragment of the mouse OX2 cDNA as a probe. The isolated genomic clone contained an insert of about 16 kB from which a 9.5 kB SalI fragment was sub-cloned into pBLUESCRIPT plasmid (Stratgene). This clone contained part of intron I, exon II (encoding the signal peptide), intron II, exon III (encoding the V-like Ig domain), intron III, exon IV (encoding the C2-like Ig domain), and part of intron IV. From this clone a targeting construct was created by replacing an NcoI fragment encoding the C-terminal part of the V-like Ig domain with the Neomycin cassette and shortening the upstream part of the clone so that it contained only the 3' part of the exon encoding the signal peptide. An ES cell line derived from C57BL/6J mice (Bruce 4; see Galli-Taliadoros, et al. (1995) J. Immunol. Methods 181:1-15 and Lemckert, et al. (1997) Nucl. Acids Res. 25:917-918) was transfected by electroporation, and G418 resistant colonies were isolated and screened for homologous recombination by PCR and Southern blotting. One homologous recombinant out of 1,000 clones was isolated and used to create chimeric mice. See Lemckert, et al. (1997) Nucl. Acids Res. 25:917-918. Male chimeras were bred with female wild type C57BL/6J mice and the offspring with black coat-color (indicating germ-line transmission) were screened for the presence of the targeted allele.~~

Please replace the paragraph on page 32, lines 7-12, as follows:

~~Compositions and methods for using mammalian ligand OX2 to treat an abnormal physiological condition in an individual. The methods comprise administering a therapeutically effective amount of OX2 alone, or in combination with other therapeutic reagents; or an OX2 antagonist. Compositions and methods are provided, for using mammalian ligand OX2 to treat an abnormal physiological condition in an~~

Appl. No. 10/086,972
Amdt. dated May 2, 2005
Reply to Office action of February 2, 2005

individual. The methods comprise administering a therapeutically effective amount of OX2 alone, or in combination with other therapeutic reagents; or an OX2 antagonist.